Neurosci. Bull. March, 2022, 38(3):327–330
https://doi.org/10.1007/s12264-021-00796-4

www.neurosci.cn
www.springer.com/12264

RESEARCH HIGHLIGHT

Loss of C9orf72 in Microglia Drives Neuronal Injury by Enhancing Synaptic Pruning in Aged and Alzheimer's Disease Mice

Mengmeng Wang[1,2] · Yuanyuan Jiang[1,2] · Zhihui Huang[1,2]

Received: 29 July 2021 / Accepted: 8 September 2021 / Published online: 16 November 2021

Many diseases are caused by the expansion of simple sequence repeats scattered throughout the human genome; they are defined as repeat expansion diseases. The size of the repeat unit ranges from trinucleotides (the vast majority) to tetranucleotides, pentanucleotides, hexanucleotides, and even dodecanucleotides [1]. Expansions of the hexanucleotide GGGGCC in the *C9orf72* gene are the most frequent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [2]. In addition to ALS and FTD, C9orf72 repeat expansions may also be associated with Alzheimer's disease (AD), since the rate of such expansions in AD cases (0.57%) is higher than normal (0.11%) [3]. However, the exact role of C9orf72 in AD is still unclear.

AD is the most common neurodegenerative disorder and has imposed an enormous economic burden on individuals and society. Most patients with AD (>95%) have a late onset (80-90 years of age), which is defined as sporadic or late-onset AD. Less than 1% of cases have familial AD at a much younger age (mean ~45 years) [4]. Currently, there are many hypotheses about the pathogenesis of AD, among which the amyloid-β hypothesis is the most influential. The core of this hypothesis is that the pathological accumulation of amyloid-β is a central event in AD pathology. Amyloid-β is overabundant due to its overproduction or reduced clearance, and it continues to oligomerize and accumulate, eventually forming plaques, which cause extensive neuronal/synaptic dysfunction and induce tau pathology [5]. Clinically, some distinctive pathological features have been observed in the clinic, such as brain atrophy, amyloid-β accumulation, neurofibrillary tangles (composed largely of hyperphosphorylated tau protein), loss of neurons and synapses, and dystrophic neurites [6]. Moreover, microglia may become activated, gather around amyloid-β plaques, and play a dual role in the onset and progression of AD [6]. Generally, microglia protect the brain by phagocytosis, and prevent AD by clearing amyloid-β aggregates and compacting amyloid-β plaques to isolate them from neurons in the early stage. However, in the late stage of AD, microglia are not sufficient to prevent its progression. Instead, they are induced into an inflammatory state because of the accumulation of toxic amyloid-β species and tau pathology. In this situation, they engulf synapses, release inflammatory mediators, and exacerbate tau pathology, all of which are detrimental to neurons [6]. Microglia-mediated synaptic loss occurs mainly *via* the complement-mediated classical pathway, in which synapses are labelled by complement component 1q (C1q) and therefore are phagocytized by microglia through complement receptor 3 on microglia [7].

McCauley and colleagues found a chronically elevated type I interferon (IFN) response in both C9orf72 ALS/FTD mice and patients due to a C9orf72 deficiency in myeloid cells [8]. Given that C9orf72 is also expressed in microglia, the resident myeloid cell in the central nervous system, it is possible that the decreased expression of C9orf72 has an impact on microglial function, and if so, how do the altered microglia affect neurodegeneration?

✉ Zhihui Huang
huang0069@hznu.edu.cn

[1] School of Pharmacy, Hangzhou Normal University, Hangzhou 311121, China

[2] Key Laboratory of Elemene Class Anti-Cancer Chinese Medicines, Engineering Laboratory of Development and Application of Traditional Chinese Medicines, Collaborative Innovation Center of Traditional Chinese Medicines of Zhejiang Province, Hangzhou Normal University, Hangzhou 311121, China

Recently, Lall and colleagues found that C9orf72$^{-/-}$ microglia display an enhanced type I IFN signature and enhanced synaptic pruning mediated by the activated microglia, leading to neuronal dysfunction and cognitive deficits in aged and AD mice [9] (Fig. 1). This study revealed that C9orf72 plays a cell-autonomous role in normal microglial function, while C9orf72$^{-/-}$ microglia lead to non-cell-autonomous dysfunction in neurons.

To investigate how to alter microglial function after the loss of C9orf72, the transcriptional profile in microglia was tested. Bulk RNA-seq and single-cell RNA-seq (scRNA-seq) were used in this study. Bulk microglia RNA-seq showed that ARM (activated response microglia) markers were decreased while IRM (interferon response microglia) markers and cytokines were elevated in aged C9orf72$^{-/-}$mice compared with aged C9orf72$^{+/+}$ and C9orf72$^{+/-}$ mice. To further determine the change of transcription, scRNA-seq was applied to microglia acutely isolated from the cortex of 12-month-old C9orf72$^{+/+}$, C9orf72$^{+/-}$, and C9orf72$^{-/-}$ mice. As expected, the results also revealed that C9orf72$^{-/-}$ mice showed gene expression with a decreased ARM and increased IRM signature. Moreover, the expression of basal stimulator of interferon gene protein and the interferon-stimulated genes CXCL10 and Mx1 were also upregulated in C9orf72$^{-/-}$ microglia, suggesting an enhanced type I IFN response. Together, these data indicate that the transcriptional signatures are changed in C9orf72-deficient microglia and accompanied by an enhanced inflammatory state.

Given the altered transcriptional signatures and the role of C9orf72 in autophagy and lysosomal function [10], researchers further found that the CD68-positive lysosomes accumulated in microglia in aged C9orf72$^{-/-}$ mice. As noted above, microglia are known to perform complement-mediated synapse pruning in both normal development and diseases. They also found that C9orf72$^{-/-}$ microglia enhanced the complement-mediated pruning of synaptic terminals in the motor cortex of aged mice with increased C1q immunoreactivity and decreased synaptic proteins. In addition, significant decreases of dendritic arborization and neurite length were detected in C9orf72$^{-/-}$ mice, morphology suggesting neuronal toxicity. To further determine the relationship between complement deposition and synapse loss, co-immunostaining of C1q and vGLUT1 (a synaptic marker) showed increased co-labeled puncta. In addition, the puncta of vGLUT1, PSD95 (a synaptic marker) with Iba1 were increased. Corresponding to the synapse loss, the aged C9orf72$^{-/-}$ mice exhibited defective spatial learning and memory with increased primary latency in Barnes maze analysis. Collectively, these results support the hypothesis that C9orf72$^{-/-}$ microglia contribute to the enhanced complement-mediated synapse elimination and neuronal deficits that result in cognitive impairment in aged C9orf72$^{-/-}$ mice.

In order to ensure that the defective dendritic morphology and changed synaptic proteins were associated with C9orf72 deficiency in microglia and not caused by C9orf72$^{-/-}$ neurons, the researchers designed a neuron-microglia co-culture system. In cortical neuronal

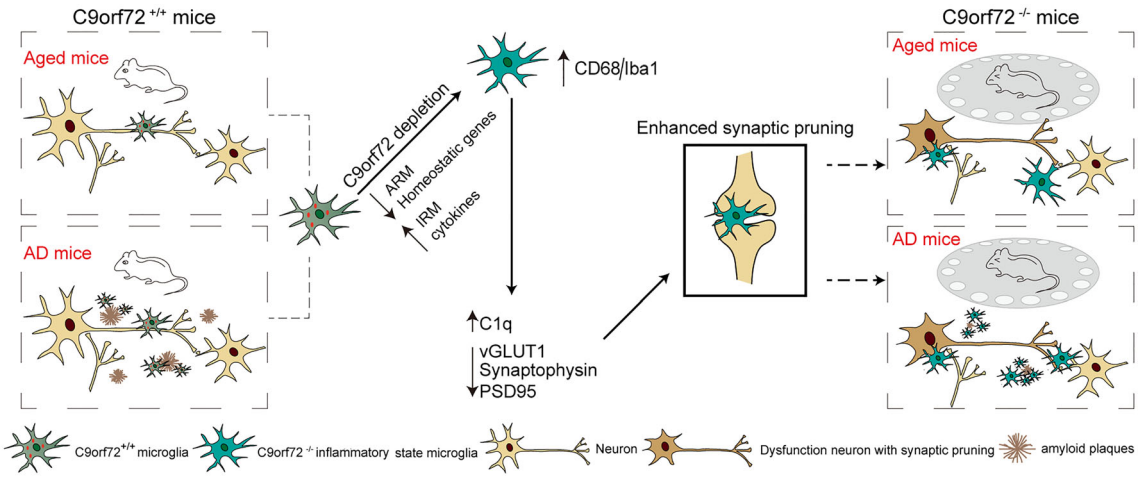

Fig. 1 C9orf72 deficiency enhances microglia-mediated synaptic pruning, leading to neuronal dysfunction and deficits in learning and memory in aged and AD mice. Bulk RNA-seq and scRNA-seq data indicate that loss of C9orf72 in microglia leads to a signature of decreased ARM and increased IRM gene expression, converting microglia to an enhanced inflammatory state characterized by CD68^{+} lysosome accumulation. Consequently, activated microglia participate in the enhanced synaptic pruning mediated by increased C1q expression. Expression of the synaptic proteins vGLUT1, synaptophysin and PSD95 are also decreased, resulting in neuronal defects with decreased dendritic arborization and total neurite length, as well as learning and memory deficits in aged or 5XFAD/C9orf72$^{-/-}$mice. In addition, plaques in 5XFAD/C9orf72$^{-/-}$mice are fewer, smaller and more compact with more microglia clustering around them, implying enhanced plaque clearance.

monocultures, there was no change in the pre- or post-synaptic proteins in C9orf72$^{+/+}$ or C9orf72$^{-/-}$ neurons. Interestingly, when C9orf72$^{+/+}$ or C9orf72$^{-/-}$ microglia were added to C9orf72$^{+/+}$ neuronal cultures, the latter showed a decreased synaptophysin density. To evaluate the cell-autonomous effects of C9orf72 loss in microglia *in vivo*, C9orf72$^{fl/fl}$:Cx3cr1^{cre+} mice, in which C9orf72 was selectively knocked out in myeloid sub-populations including microglia, was used in this study. The results showed that 12-month-old C9orf72$^{fl/fl}$:Cx3cr1^{cre+} mice recapitulated the synaptic phenotypes found in C9orf72$^{-/-}$ mice, such as CD68^{+} lysosome accumulation in microglia, increased C1q expression, and decreased synaptic protein synaptophysin. In summary, these results demonstrate that C9orf72 loss in microglia have cell-autonomous, enhanced phagocytic activity and induce a non-cell-autonomous synapse loss in neurons.

Besides the ALS/FTD phenotype, C9orf72 repeat expansions are also associated with AD syndromes. To assess the effect of C9orf72 deficiency on amyloid-β pathology, C9orf72$^{-/-}$ mice were crossed with 5XFAD transgenic mice, a model of amyloid-β deposition, in which amyloid-β plaques begin to accumulate after 3 months and the plaque burden is large after 6 months. The results showed that 5XFAD/C9orf72$^{-/-}$ mice had less amyloid-β plaque accumulation in cortical and hippocampal regions compared with the other genotypes at 6 months. Meanwhile, there was no difference in the numbers of Iba1-positive microglia across the different genotypes. However, the plaques in 5XFAD/C9orf72$^{-/-}$mice were smaller and more compact with more microglia clustering around them. They also proposed that circulating autoantibodies against amyloid-β may contribute to microglia-mediated plaque clearance. Taken together, these results illustrate that C9orf72$^{-/-}$ microglia engage with and enclose amyloid-β plaques more effectively and attenuate their accumulation.

Hong *et al.* showed that, in mouse models, complement- and microglia-mediated synaptic loss occurs before overt plaque deposition in AD [11]. Therefore, the researchers then investigated how C9orf72$^{-/-}$ microglia affect synaptic integrity during 5XFAD plaque accumulation. Because amyloid-β plaques only accumulated in 4-month-old 5XFAD mice with no neuronal loss, they assessed the expression of the presynaptic proteins synaptophysin and vGLUT1, and the postsynaptic marker PSD95 in 4-month-old 5XFAD/C9orf72$^{-/-}$ mice; these results showed that both of them were reduced. Moreover, they found significant reductions in cortical dendritic arborization and total neurite length, and increased lysosome accumulation in 5XFAD/C9orf72$^{-/-}$ mice. Increased C1q^{+}:vGLUT1^{+} puncta along with enhanced vGLUT1^{+} and PSD95^{+} puncta in Iba1^{+} microglia were likewise found in 5XFAD/C9orf72$^{-/-}$ mice, which confirmed that enhanced synaptic loss is driven by complement-mediated pruning. In the end, the Barnes maze test was applied to determine if there is a behavioral correlate of the enhanced early synapse loss in 5XFAD/C9orf72$^{-/-}$ mice. The results suggested that C9orf72 deficiency exacerbates the spatial learning and memory defects in 5XFAD/C9orf72$^{-/-}$ mice. In conclusion, it is very interesting that the altered functional status of C9orf72$^{-/-}$ microglia in an AD mouse model inhibits the growth of extracellular amyloid-β plaques while paradoxically also enhancing the synaptic loss, memory deficits, and neuronal damage.

In summary, Lall and colleagues showed that a deficiency of C9orf72 triggers microglia into an inflammatory state that promotes complement-mediated synaptic loss and functional impairment of neurons, which in turn leads to cognitive impairment in aged or AD mice. This study identified the role of C9orf72 in modulating microglial-mediated synaptic engulfment and provides a clinical reference for the treatment of neurodegenerative diseases in repeat expansion carriers. More importantly, the group pointed out that distinctive neurodegeneration is exhibited in aged C9orf72$^{-/-}$ mice, such as aberrant neuronal morphology, increased complement-mediated cortical synaptic loss, and learning and memory deficits that prior studies did not demonstrate. Besides, as noted above, amyloid-β deposits in the brain have been the leading scientific hypothesis for AD. Yet some scholars still believe that amyloid-β may not be a reason, but rather may be a feature accompanying AD, given the failure of clinical treatments targeting amyloid-β and other findings [12]. This study provides evidence for the latter viewpoint, as neuronal dysfunction and cognitive deficits were found in 5XFAD/C9orf72$^{-/-}$ mice along with fewer, smaller, and more compact plaques. However, several issues still need to be further studied before clinical testing. Given the differences between human beings and animals, there is no exact answer to whether the loss of C9orf72 expression in human microglia is sufficient to alter their transcriptional profile like the aged animals. In addition, considering that peripheral IFNα can also induce unique genetic and phenotypic changes in microglia, and result in aberrant synaptic pruning in neuropsychiatric disease [13], this study does not exclude the possibility that loss of C9orf72 in other cells such as peripheral myeloid cells may have an effect on microglial phenotypes due to total C9orf72$^{-/-}$ knockout. Furthermore, we note that C9orf72$^{-/-}$ mice were crossed with 5XFAD transgenic mice, a model of familial AD, to explore the behavior of C9orf72$^{-/-}$ microglia in AD. Does the same phenotype occur in the mouse model of sporadic AD (the most common type) with C9orf72 deficiency? Finally, since each cell plays its unique role in the normal operation of the body, it is not clear whether C9orf72-knockout mice generate other unknown effects.

For example, is the function of astrocytes or oligodendrocytes in C9orf72$^{-/-}$ mice also affected? And what about the survival or lifespan of C9orf72$^{-/-}$ mice? Clarifying these uncertainties may require continuous exploration. In the long run, this study provides a new perspective for understanding the pathological mechanism of the disease and creates a new platform for AD treatment.

Acknowledgements This Research Highlight was supported by the National Natural Science Foundation of China (92049104 and 82103210) and the Research Start-up Project by Hangzhou Normal University (4125C5021920453 and 4125C50220204106).

References

1. Goodman LD, Bonini NM. New roles for canonical transcription factors in repeat expansion diseases. Trends Genet 2020, 36: 81–92.
2. Hao ZB, Wang R, Ren HG, Wang GH. Role of the C9ORF72 gene in the pathogenesis of amyotrophic lateral sclerosis and frontotemporal dementia. Neurosci Bull 2020, 36: 1057–1070.
3. Harms M, Benitez BA, Cairns N, Cooper B, Cooper P, Mayo K, *et al*. C9orf72 hexanucleotide repeat expansions in clinical Alzheimer disease. JAMA Neurol 2013, 70: 736–741.
4. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. Nat Rev Dis Primers 2015, 1: 15056.
5. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 2016, 8: 595–608.
6. Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. J Cell Biol 2018, 217: 459–472.
7. Stephan AH, Barres BA, Stevens B. The complement system: An unexpected role in synaptic pruning during development and disease. Annu Rev Neurosci 2012, 35: 369–389.
8. McCauley ME, O'Rourke JG, Yáñez A, Markman JL, Ho R, Wang XC, *et al*. C9orf72 in myeloid cells suppresses STING-induced inflammation. Nature 2020, 585: 96–101.
9. Lall D, Lorenzini I, Mota TA, Bell S, Mahan TE, Ulrich JD, *et al*. C9orf72 deficiency promotes microglial-mediated synaptic loss in aging and amyloid accumulation. Neuron 2021, 109: 2275–2291.e8.
10. Burk K, Pasterkamp RJ. Disrupted neuronal trafficking in amyotrophic lateral sclerosis. Acta Neuropathol 2019, 137: 859–877.
11. Hong S, Beja-Glasser VF, Nfonoyim BM, Frouin A, Li SM, Ramakrishnan S, *et al*. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science 2016, 352: 712–716.
12. Ayton S, Bush AI. β-amyloid: The known unknowns. Ageing Res Rev 2021, 65: 101212.
13. Aw E, Zhang YY, Carroll M. Microglial responses to peripheral type 1 interferon. J Neuroinflammation 2020, 17: 340.